ESSENTIAL GUIDES TO METHOD DEVELOPMENT IN GAS CHROMATOGRAPHY

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Introduction

Separations are possible in gas chromatography if the solutes differ in their vapour pressure and/or intensity of solute–stationary–phase interactions. As a minimum requirement the sample, or some convenient derivative of it, must be thermally stable at the temperature required for vaporization. The fundamental limit for sample suitability is established by the thermal stability of the sample and system suitability by the thermal stability of column materials. In contemporary practice an upper temperature limit of about 425°C and a sample molecular weight less than 1250 is indicated with only minor exceptions. A large number of general and selective derivatizing reagents are available for sample modification to enhance compound thermal stability, improve sample separation properties, and to provide compound-selective detection.

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Column Types

Wall-coated open tubular columns (WCOT columns), or simply capillary columns, and classical packed columns dominate the practice of gas–liquid chromatography. Porous layer open tubular columns (PLOT columns) and classical packed columns dominate the practice of gas–solid chromatography. Classical packed columns are usually 0.5–3 m long with an internal diameter greater than 2 mm packed with adsorbent or liquid-coated support particles of 100–250 μm diameter. WCOT columns are typically up to 100 m long with internal diameters of capillary dimensions coated with a thin, and usually immobilized, film of stationary phase leaving an open interior passageway down the centre of the column. PLOT columns are identical to WCOT columns with the liquid phase replaced by a layer of fine adsorbent particles. WCOT and PLOT columns are the first choice for analytical separations because of their superior peak capacity and greater chemical inertness. Packed columns offer a lower cost choice for some applications, are easier to use, are relatively tolerant of thermally unstable and involatile sample components and are better suited to isolating preparative-scale quantities of materials. Only a limited number of poly(siloxane) and poly(ethylene glycol) stationary phases have been successfully immobilized in WCOT columns compared to the larger number and variety of stationary phases available for use in packed columns.

Column Properties

The high permeability of WCOT columns allows long columns to be used to provide very high total plate numbers, as indicated in **Table 1**. Narrow-bore and thin-film columns are intrinsically the most efficient and are selected for fast chromatography. Since resolution increases only as the square root of the plate number, and also the column length, large values for the plate number are required for difficult separations. Such large numbers are available in gas chromatography, albeit at the expense of separation time, allowing separations to be achieved with only minimal differences in selectivity. In contrast to other chromatographic methods, separations in gas chromatography are often achieved through kinetic optimization, allowing many separations to be obtained on a limited number of stationary phases. A favourable feature of kinetic optimization is that the outcome is readily predictable from simple arithmetic calculations once some information of peak order has been established in a trial separation.

At a given temperature the partition coefficient is constant and the observed retention factor will depend on the phase ratio. The phase ratio is given by the column volume accessible to the mobile phase divided by the volume of stationary phase. Columns with a large phase ratio provide small retention factors for volatile compounds and require inconveniently large plate numbers to provide adequate resolution. Columns with a low phase ratio, that is, thick film columns, have a lower intrinsic efficiency than thin film columns, but provide better resolution of volatile compounds, because they provide more favourable retention factors. They also allow separations of volatile compounds at a higher and more convenient temperature range than is possible with thin film columns. For volatile compounds this often means at temperatures above room temperature as opposed to cryogenic temperatures. For high boiling compounds, columns with a low phase ratio are not

Table 1 Characteristic properties of some representative columns

Column type	*Length (m)*	*Internal diameter (mm)*	*Film thickness (μm)*	*Phase ratio*	*Column plate number*	*Plates per metre*
Classical packed	2	2.16	10%(w/w)	12	3640	1820
	2	2.16	5%(w/w)	26	4000	2000
WCOT	30	0.10	0.10	249	480000	16000
	30	0.10	0.25	99	368550	12285
	25	0.25	0.25	249	160000	6400
	50	0.25	0.25	249	320000	6400
	100	0.25	0.25	249	640000	6400
	30	0.32	0.32	249	150000	5000
	30	0.32	0.50	159	131330	4380
	30	0.32	1.00	79	102080	3400
	100	0.32	1.00	79	304200	3400
	30	0.32	5.00	15	68970	2300
	10	0.53	1.00	132	23500	2340
	30	0.53	1.00	132	70420	2340
	10	0.53	5.00	26	14700	1470
	30	0.53	5.00	26	43940	1470
	50	0.53	5.00	26	73200	1470

useful because they lead to long separation times. Increasing the phase ratio by reducing the film thickness lowers the retention factors to a value within the optimum range so that there is little deterioration in resolution and faster separations are obtained. Packed columns have low phase ratios compared to most WCOT columns. For compounds of moderate and low volatility, separation times on packed columns are generally longer. Since several combinations of film thickness and column radius can be used to generate the same phase ratio, there are other factors that need to be considered for selecting these variables for a particular separation.

Mobile-Phase Selection

Nearly all separations are achieved with hydrogen, helium or nitrogen as the carrier gas. At temperatures and pressures typical of normal operation in gas chromatography these gases behave almost ideally, providing a transport mechanism for the sample without influencing selectivity. The exception is gas–solid chromatography where the carrier gas participates in the retention process through competition with the sample for stationary-phase adsorption sites. Differences between hydrogen, helium and nitrogen are not usually large but absolute retention and retention order can change as a function of the carrier gas type and average carrier gas pressure. Heavier carrier gases, such as carbon dioxide, are more effective at influencing retention in gas–solid chromatography than the common carrier gases.

Although the choice of carrier gas does not significantly influence selectivity in gas–liquid chromatography, it can still influence resolution through its effect on efficiency. This results from differences in gas diffusivity. The separation time is also affected because the optimum carrier gas velocity decreases with solute diffusion rates. In pressure-limiting conditions, gas viscosity differences are important as well. Nitrogen provides lower plate heights but at a lower optimum velocity (**Figure 1**), leading to long separation times. Close to the optimum plate height region, the ascending portions of the curves are shallower for hydrogen and helium. Thus, for separations at mobile-phase velocities higher than the optimum velocity, hydrogen and, to a lesser extent, helium provide faster separations than nitrogen with little loss in efficiency. For thick-film columns (> 0.5 μm), diffusion in the stationary phase is a significant factor in zone broadening and the relative contribution of the carrier gas to separation performance and time are not as great. Thick-film columns should be operated close to the optimum velocity, with the choice of carrier gas being less significant. Nitrogen is often the preferred carrier gas for these columns. For packed columns nitrogen provides (slightly) higher efficiency at low temperatures and flow rates, while hydrogen is superior at higher temperatures and at above optimum velocities. Hydrogen is preferred in pressure-limited conditions because of its lower viscosity. A considerable difference in the relative cost of helium in the USA and Europe has resulted in different preferences on the two continents. For WCOT columns, helium is widely used in the USA for safety rather than theoretical considerations, while hydrogen is commonly used in Europe.

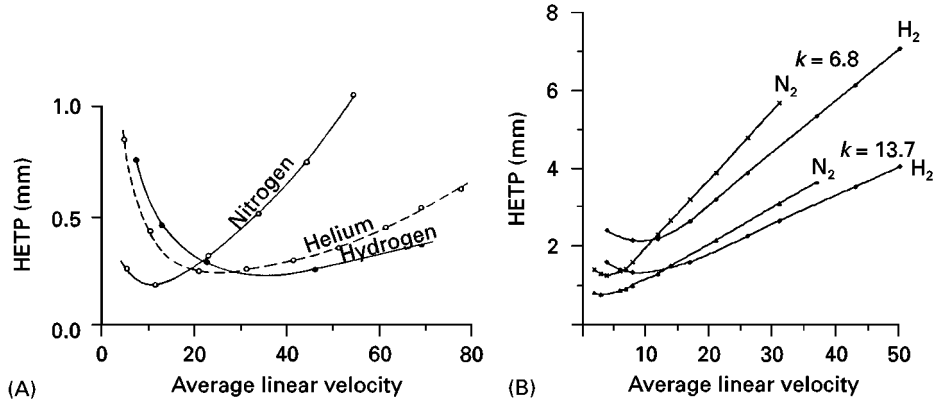

Figure 1 Influence of the choice of carrier gas on the efficiency of (A) a thin-film and (B) a thick-film WCOT column (*k* is the retention factor).

Stationary-Phase Selection

Given the nonsolvating properties of the mobile phase in gas chromatography, selectivity optimization is a matter of stationary-phase selection. Over the years thousands of substances have been evaluated as stationary phases and most abandoned in favour of a smaller number of liquids and adsorbents with favourable temperature operating ranges, kinetic properties and possibility of immobilization if used in WCOT columns.

Packed-column liquid phases can be roughly categorized into four groups:

1. hydrocarbon and perfluorocarbon liquid phases
2. ether and ester liquid phases
3. liquid organic salts
4. poly(siloxane) liquid phases

Representative examples and their useful temperature operating range are summarized in **Tables 2** and **3**. Most can be considered useful for general applications, except for the perfluorocarbon liquid phases that are used for the speciation of perfluorocarbon compounds or the separation of reactive compounds (metal fluorides, interhalogen compounds and nonmetal halides) that tend to destroy conventional phases. The family of poly(siloxanes) provides the widest range of favourable stationary-phase properties and variation in selectivity. They are the most widely used stationary phases for packed-column

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Table 2 Characteristic properties of some liquid phases used in packed-column gas chromatography

Name	*Structure*	*Temperature range* (°C)	
		Miminum	*Maximum*
Hexadecane	$C_{16}H_{34}$	< 20	50
Squalane	2,6,10,15,19,23-hexamethyltetracosane	< 20	120
Apolane-87	$(C_{18}H_{37})_2CH(CH_2)_4C(C_2H_5)_2(CH_2)_4CH(C_{18}H_{37})_2$	30	280
Fomblin YR	$-[OCF(CF_3)CF_2)_n(OCF_2)_m]-$	30	< 255
PPE-5	$C_6H_5O(C_6H_5O)_3C_6H_5$	20	200
Dioctyl phthalate	$C_6H_4(COOC_8H_{17})_2$	< 20	160
EGS	$HO(CH_2)_2[OOCCH_2CH_2COO(CH_2)_2]_nOH$	100	210
DEGS	$HO(CH_2)_2O(CH_2)_2[OOCCH_2CH_2COO(CH_2)_2O(CH_2)_2]_nOH$	20	200
Carbowax 20M	$HO(CH_2CH_2O)_nCH_2CH_2OH$	60	225
FFAP		50	250
1,2,3-Tris(2-cyanoethoxy)propane	$(CH_2OCH_2CH_2CN)_3$	20	170
Tetrabutylammonium perfluorooctanesulfonate		< 20	220
Tetrabutylammonium 4-toluenesulfonate		55	200
Tetrabutylammonium tetrafluoroborate		162	290
Ethylammonium 4-toluenesulfonate		121	220
Tetrabutylphosphonium chloride		83	230

PPE-5, Poly(phenyl ether); EGS, poly(ethylene glycol succinate); DEGS, poly(diethylene glycol succinate); Carbowax 20M, poly(ethylene glycol); FFAP, Carbowax 20M treated with 2-nitroterephthalic acid.

Table 3 Characteristic properties of some poly(siloxane) liquid phases used for packed-column gas chromatography

Name	*Structure*	*Temperature operating range* (°C)	
		Minimum	*Maximum*
OV-1	Dimethylsiloxane (gum, molecular weight $> 10^6$)	100	350
OV-101	Dimethylsiloxane (oil, molecular weight 3×10^4)	< 20	350
OV-7	Phenylmethyldimethylsiloxane 80% methyl and 20% phenyl	< 20	350
OV-17	Phenylmethylsiloxane 50% methyl and 50% phenyl	< 20	350
OV-25	Phenylmethyldiphenylsiloxane 25% methyl and 75% phenyl	< 20	300
OV-210	Trifluoropropylmethylsiloxane 50% methyl and 50% 3,3,3-trifluoropropyl	< 20	275
OV-225	Cyanopropylmethylphenylmethylsiloxane 50% methyl, 25% phenyl and 25% 3-cyanopropyl	< 20	250
Silar 7CP	Cyanopropylphenylsiloxane 75% 3-cyanopropyl and 25% phenyl	50	250
OV-275	Di(cyanoalkyl)siloxane 70% 3 cyanopropyl and 30% 2-cyanoethyl		250
Silar 10CP	Di(3-Cyanopropyl)siloxane	50	250

gas–liquid chromatography and, because of their ease of immobilization, are the dominant stationary phases used to prepare WCOT columns (**Table 4**). With today's technology the only other family of stationary phases that can be immobilized for WCOT columns are the poly(ethylene glycols).

The selectivity of the stationary phases is of more interest than their chemical structure for method development. Liquid stationary phases have been classified based on their solvent strength (polarity) and selectivity. Classification based on the idea of polarity has had to be abandoned because of the lack of a working definition. Selectivity is defined as the relative capacity of a stationary phase for specific intermolecular interactions, such as dispersion, induction, orientation and complexation (including hydrogen bond formation). Unlike solvent strength (polarity) it should be feasible to devise experimental scales of stationary-phase selectivity. Modern approaches to stationary-phase classification by selectivity are based on the cavity model of solvation. This model assumes that the transfer of a solute from the gas phase to solution in the stationary phase involves three steps. Initially a cavity is formed in the stationary phase of the same size as the solute. The solute is then transferred to the cavity with reorganization of solvent molecules around the cavity and the set-up of solute–solvent interactions. Retention in gas–liquid chromatography, therefore, will depend on the cohesive energy of the stationary phase, represented by the

Table 4 Rough guide to the temperature operating range for bonded poly(siloxane) liquid phases in open tubular columns

Type	*Temperature range* (°C)		*High temperature version*
	Minimum	*Maximum*	
Dimethylsiloxane	− 60	325	420
Dimethyldiphenylsiloxane (5% diphenyl)	− 60	325	420
Dimethyldiphenylsiloxane (35% diphenyl)	40	300	340
Dimethyldiphenylsiloxane (50% diphenyl)	40	325	390
Methylphenylsiloxane	0	280	
Dimethyldiphenylsiloxane (65% diphenyl)	50	260	370
3,3,3-Trifluoropropylmethylsiloxane (50% trifluoropropyl)	45	240	300
3-Cyanopropylphenyldimethylsiloxane (6% cyanopropylphenyl and 84% dimethyl)	20	280	
3-Cyanopropylphenyldimethylsiloxane (25% cyanopropylphenyl and 75% dimethyl)	40	240	
3-Cyanopropylphenyldimethylsiloxane (50% cyanopropylphenyl and 50% dimethyl)	40	230	
3-Cyanopropyl-silphenylene co-polymer (equivalent to 70% dicyanopropyl)			290
Poly(ethylene glycol)	20	250	280
FFAP	40	250	

FFAP, Poly(ethylene glycol) treated with 2-nitroterephthalic acid.

free energy required for cavity formation, the formation of additional dispersion interactions of a solute–solvent type, and on selective solute–solvent polar interactions dependent on the complementary character of the polar properties of the solute and stationary phase. Quantitatively, these interactions are described by the solvation parameter model set out below in the form suitable for stationary-phase classification:

$$\log k = c + rR_2 + s\pi_2^{\mathrm{H}} + a\Sigma\alpha_2^{\mathrm{H}} + b\Sigma\beta_2^{\mathrm{H}} + l \log L^{16} \quad [1]$$

where k is the retention factor. The remainder of the equations is made up of product terms called system constants (r, s, a, b, l) and solute descriptors (R_2, π_2^{H}, $\Sigma\alpha_2^{\mathrm{H}}$, $\Sigma\beta_2^{\mathrm{H}}$, $\log L^{16}$). Each product term represents a contribution from a defined intermolecular interaction to the solute property. The solute descriptors are free energy-related solute properties known for about 4000 compounds with others available by estimation or from experiment. They are not of immediate interest to us here except to note that once the system constants are established, the retention property of any solute with known or easily estimated solute descriptors can be estimated for that system by simple arithmetic calculation using the model described. The system constants (also called phase constants in gas chromatography) contain the information of the stationary-phase properties and provide an unambiguous means of classification. The r phase constant refers to the ability of the stationary phase to interact with solute n– or π–electron pairs. The s phase constant to the ability of the stationary phase to take part in dipole-type interactions. The a phase constant is a measure of stationary-phase hydrogen bond basicity and the b phase constant a measure of stationary-phase hydrogen bond acidity. The l phase constant describes (in part) the contribution of cavity formation and dispersion interactions to retention and, more specifically, indicates the ability of the stationary phase to separate members of a homologous series. The phase constants for any stationary phase can be determined through the method of multiple linear regression analysis by measurement of a retention property for a series of varied solutes with known solute descriptors.

The stationary phase constants for a number of common liquid phases at a reference temperature of 121°C are summarized in **Table 5**. The system constants in Table 5 are only loosely scaled to each other so that changes in magnitude in any column can be read directly, but changes in magnitude along rows must be interpreted more cautiously. Most stationary phases possess some capacity for lone-pair electron interactions (r constant), but selectivity for this interaction is all but nonexistent among common stationary phases. Fluorine-containing stationary phases have negative values of the r constant representing the tighter binding of electron pairs in fluorocarbon compared to hydrocarbon groups. Lone pair electron interactions do not usually make a significant contribution to retention in gas–liquid chromatography and are not considered a primary means of selectivity optimization. The most striking feature of Table 5 is the paucity of stationary phases with significant hydrogen bond acidity (b constant). In the case of EGAD, DEGS and TCEP, the small b phase constants indicated in Table 5 are probably a product of impurities and thermal modification of the stationary phases during use rather than a fundamental property of the stationary phases themselves.

A few novel stationary phases with strong hydrogen bond acid properties have been synthesized recently, but none of these are commercially available. Stationary-phase hydrogen bond acid interactions, therefore, do not contribute significantly to method development strategies for the commonly used stationary phases. The only practical exception seems to be the poly(trifluoropropylmethylsiloxane) WCOT column stationary-phase DB-210, which exhibits some weak hydrogen bond acidity, presumably acquired through the immobilization process that is absent from the structurally similar packed-column stationary phase QF-1. That leaves the most important stationary-phase properties for selectivity optimization as their cohesive energy and capacity for dipole-type and hydrogen bond base interactions.

Cluster analysis provides a visual picture of the difference in selectivity for different stationary phases and a classification of their properties into groups of similar selectivity (**Figure 2**). The stationary phases most similar to each other are next to each other and are connected. Connections at the extreme left-hand side of the dendrogram occur for phases with similar properties and those towards the right-hand side with greater degrees of difference. Stationary phases with no paired descendents are singular phases with properties that cannot be duplicated by the other phases. The stationary phases are classified into five groups with three phases behaving independently. Group 1 contains squalane, Apolane-87, OV-3. OV-7, SE-30 and OV-105. These are phases of low cohesive energy with minimal capacity for polar interactions. The second group of stationary phases contains OV-22, OV-25, OV-11, OV-17, PPE-5 and DDP. These phases have low cohesive energy and are weakly dipolar and hydrogen bond basic. QF-1 is loosely connected to this group but is significantly

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Table 5 System constants derived from the solvation parameter model for common stationary phases at 121°C

Stationary phase	*System constant*				
	r	*s*	*a*	*b*	*l*
Hydrocarbon phases					
Squalane	0.13	0.01	0	0	0.58
Apolane-87	0.17	0	0	0	0.56
Ether and ester phases					
Poly(phenyl ether) 5 rings (PPE-5)	0.23	0.83	0.34	0	0.53
Carbowax 20M (CW20M)	0.32	1.26	1.88	0	0.45
Poly(ethylene glycol) Ucon 50 HB 660	0.37	0.63	1.28	0	0.50
Nitroterephthalic acid modified poly(ethylene glycol) (DB-FFAP)	0.21	1.42	2.08	0	0.43
1,2,3-Tris(2-cyanoethoxypropane) (TCEP)	0.12	2.09	2.10	0.26	0.37
Didecylphthalate (DDP)	0	0.75	0.77	0	0.56
Poly(ethylene glycol adipate) (EGAD)	0.13	1.39	1.82	0.21	0.43
Poly(diethylene glycol succinate) (DEGS)	0.23	1.57	2.11	0.17	0.41
Liquid organic salts					
Tetrabutylammonium 4-toluenesulfonate (QBApTS)	0.16	1.58	3.30	0	0.46
Tetrabutylammonium tris(hydroxymethyl)methyl-amino-2-hydroxy-1-propanesulfonate (QBATAPSO)	0.27	1.96	3.06	0	0.32
Tetrabutylammonium 4-morpholinepropanesulfonate (QBAMPS)	0	1.75	3.54	0	0.55
Tetrabutylammonium methanesulfonate (QBAMES)	0.33	1.45	3.76	0	0.44
Poly(siloxane) phases					
Poly(dimethylsiloxane) (SE-30)	0.02	0.19	0.13	0	0.50
Poly(dimethyldiphenylsiloxane) (DB-5) (5 mol% diphenylsiloxane)	0	0.28	0.19	0	0.51
Poly(dimethylmethylphenylsiloxane) (OV-3) (10 mol% phenyl)	0.03	0.33	0.15	0	0.50
Poly(dimethylmethylphenylsiloxane) (OV-7)	0.06	0.43	0.17	0	0.51
Poly(dimethylmethylphenylsiloxane) (OV-11) (35 mol% phenyl)	0.10	0.54	0.17	0	0.52
Poly(methylphenylsiloxane) (OV-17)	0.07	0.65	0.26	0	0.52
Poly(dimethyldiphenylsiloxane) (HP-50) (50 mol% diphenylsiloxane)	0.16	0.62	0.28	0	0.47
Poly(methylphenyldiphenylsiloxane) (OV-22) (65 mol% phenyl)	0.20	0.66	0.19	0	0.48
Poly(methylphenyldiphenylsiloxane) (OV-25)	0.28	0.64	0.18	0	0.47
Poly(cyanopropylmethyldimethylsiloxane) (10 mol% cyanopropylmethylsiloxane) (OV-105)	0	0.36	0.41	0	0.50
Poly(cyanopropylmethylphenylmethylsiloxane) (OV-225)	0	1.23	1.07	0	0.47
Poly(cyanopropylphenyldimethylsiloxane) (50 mol% cyanopropylphenylsiloxane) (DB-225)	0	1.21	1.18	0	0.44
Poly(dicyanoalkylsiloxane) (OV-275)	0.21	2.08	1.99	0	0.29
Poly(trifluoropropylmethylsiloxane) (QF-1)	−0.45	1.16	0.19	0	0.42
Poly(trifluoropropylmethylsiloxane) (DB-210)	−0.27	1.15	0	0.19	0.43
Poly(dimethylsiloxane)-poly(ethylene glycol) copolymer (OV-330)	0.10	1.06	1.42	0	0.48

more dipolar and has a more significant and opposite capacity for lone pair electron interactions. The third group contains OV-330 and OV-225 with UH50B loosely connected to this group. Compared to the second group these stationary phases are more dipolar and hydrogen bond basic and slightly more cohesive. They represent an increase in the intensity of the same range of interactions as the group 2 stationary phases. The fourth group contains the liquid organic salts with QBATAPSO distinguished within this group by its greater cohesive energy. Phases in this group are dipolar and the strongest hydrogen bond bases. The fifth group of solvents is divided into two subgroups. TCEP and OV-275 are strongly dipolar, hydrogen bond basic and have high cohesive energy. EGAD, CW20M and DEGS have a similar range of polar interactions, but not quite as intense, and have a lower cohesive energy. For selectivity optimization in packed-column gas chromatography, a single phase is initially selected from each group. Subsequently, for fine-tuning additional phases are selected from within the group, identified as possessing the desired separation properties.

For historical reasons stationary phases are classified at a common reference temperature of about 120°C. The capacity of a stationary phase for specific intermolecular interactions determined at one temperature can be misleading for selectivity optimization at other distant temperatures. The broad outlines in Table 5 and Figure 2 remain true but changes in

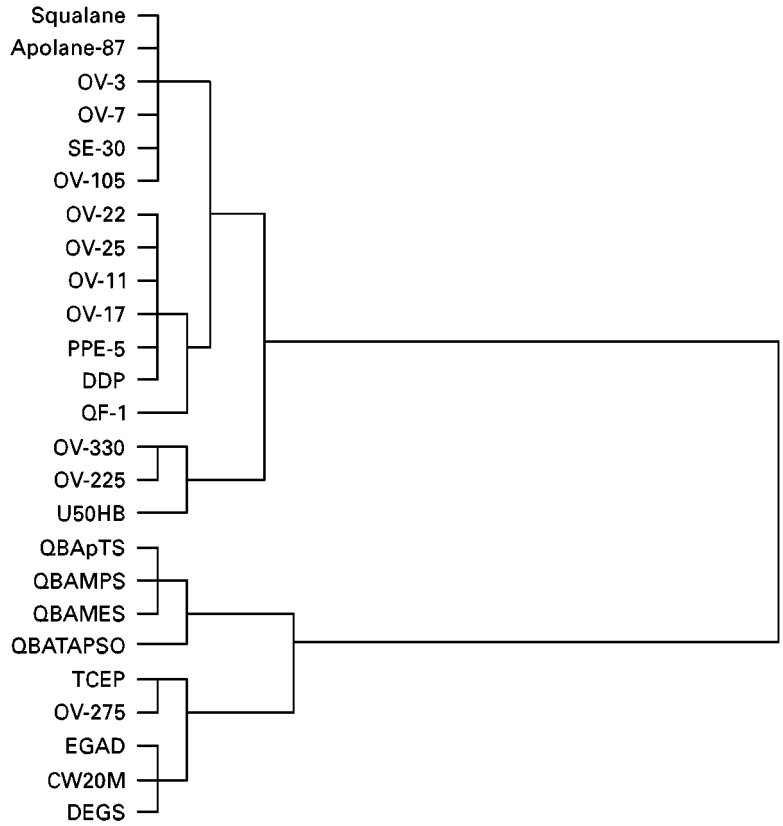

Figure 2 Nearest neighbour complete link cluster dendrogram for some common stationary phases. The abbreviations for the stationary phases are identified in Tables 2, 3 and 5.

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rank order due to cross-overs occur at different temperatures. Also, selectivity differences between individual stationary phases are enhanced at low temperatures with phases becoming more alike at higher temperatures. Information on the contribution of polar interactions to retention at high temperatures is scarce. These contributions could be small and stationary-phase selectivity differences rather limited at high temperatures.

Gas–solid chromatography is used for a narrower range of separations than gas–liquid chromatography. Because of higher retention, typical applications are the separation of fixed gases, volatile hydrocarbons, halocarbons, organic solvents and sulfur gases. The presence of immobilized active centres enhances the separation of isomers and isotopes. These separations are often difficult or impossible with liquid phases. A rough guide to the selection of sorbents for particular applications is given in **Table 6**. PLOT columns provide higher efficiency, faster separations and faster column regeneration compared to packed columns. Surface coating with inorganic salts and small amounts of liquid phase are used to extend the molecular weight separation range with inorganic oxide and carbon sorbents and to optimize selectivity. PLOT columns generally require greater care in their use than WCOT columns. Other features include lower efficiency than WCOT columns, limited sample capacity and high activity.

General Elution Problem

In gas chromatography there is an approximate exponential relationship between retention time and solute boiling point at a constant (isothermal) column temperature. Consequently, it is impossible to establish a suitable compromise temperature for the separation of mixtures with a boiling point range exceeding about 100°C. This is generically referred to as 'the general elution problem' and is characterized by long separation times, poor separations of early eluting peaks and poor detectability of late eluting peaks due to zone broadening. The general solution to this problem is the use of programmed temperature and flow separation modes. Neither constant nor programmed modes are superior to each other. They are complementary, with the properties of the sample deciding which approach is adopted.

Temperature programming is the most popular programmed separation mode in gas chromatography.

Table 6 General applications of PLOT columns in gas chormatography

Stationary phase	*Maximum operating temperature* (°C)	*Typical applications*
Alumina oxide	200	Alkanes, alkenes, alkynes and aromatic hydrocarbons from C_1 to C_{10}. C_1 and C_2 halocarbons
Silica gel	250	Hydrocarbons (C_1 to C_4), inorganic gases, volatile ethers, esters and ketones
Carbon	350	Inorganic gases and hydrocarbons (C_1 to C_5)
Carbosieves	150	C_1 to C_6 compounds
Molecular sieves (5A and 13X)	350	Hydrogen, oxygen, nitrogen, methane and noble gases Hydrocarbons (C_1 to C_3) on 5A with higher alkanes on 13X (up to C_{12}) but not isomer separations
Porous polymers		
Q	310	Hydrocarbons (C_1 to C_{14}), halocarbons (C_1 and C_2), volatile oxygenated solvents (C_1 to C_6), thiols, amines, nitro compounds, nitriles, water and inorganic gases
S	250	
U	190	

Q, Poly(divinylbenzene-styrene); S, poly(divinylbenzene-vinylpyridine); U, poly(divinylbenzene-ethylene glycol dimethacrylate).

Stationary phases of high thermal stability allow wide temperature ranges to be used and temperature is easily adjusted and controlled. Temperature programme techniques are the most useful approach for scouting the properties of an unknown sample and are compatible with the large volume injection modes employing low temperature solute refocusing used in trace analysis. Flow programming is easily achieved with instruments fitted with electronic pressure control but is limited by the narrow pressure range which is usually available. It can be used to separate thermally labile compounds at a lower temperature than required for temperature-programmed separations. On the other hand, flow programming results in a loss of efficiency for late eluting peaks and presents difficulties in calibrating flow-sensitive detectors.

A temperature programme consists of a series of changes in the oven temperature and includes isothermal and controlled temperature rise segments. In essence, most programmes are simple, consisting of an initial isothermal period, a linear temperature rise segment, final isothermal period at the temperature reached at the end of the rise segment, and a cool-down period to return the oven to the starting temperature. The initial and final isothermal periods are optional, the temperature rise segment can be selected over a wide range (0.1 to *c.* $100^{\circ}C\,min^{-1}$), nonlinear changes in temperature may be used (extremely rare) and for complex mixtures, several linear programmes may be used in sequence to optimize the separation. The initial oven temperature is selected with due consideration to the resolution of the earliest eluting peaks in the chromatogram. If the temperature chosen is too high, the resolution of the initial peaks may be inadequate and, if it is too low, resolution may be acceptable but the separation time will be extended needlessly. The final temperature should be selected so that the termination of the temperature rise segment and elution of the last sample component coincide unless the last few eluting peaks are particularly difficult to separate and require an isothermal period. Peaks eluting after completion of the temperature rise segment will be wider than those eluted during the programme. The selection of the heating rate represents a compromise between the necessity of maintaining a minimum acceptable resolution for the sample and the desire to reduce the separation time. This is governed largely by the complexity of the sample and its boiling point range. For samples containing components of different polarity temperature-induced changes in selectivity make the prediction of the resolution of closely spaced peaks a problem. Certain generalities can be made however. For the most difficult separations a slow heating rate will usually provide the optimum resolution. The separation time of weakly retained solutes is more readily adjusted by changing the flow rate of the carrier gas than the heating rate. For strongly retained solutes increasing the heating rate causes a proportional decrease in the separation time at a constant carrier gas flow rate. The retention time of well-retained solutes are less affected by changing flow rates in temperature-programmed gas chromatography.

The lack of an exact mathematical model to describe temperature-programmed separations makes computer simulation for their rapid optimization difficult. Simplex optimization of experimental variables and a model based on the linear elution strength approximation have been used with some success. The linear elution strength approach has the advantage that it only requires experimental data from two temperature-programmed separations of a sample using different programme rates. A series

of empirical equations are then employed to predict optimum separation conditions using relative resolution maps.

Generic Method Development

Method development commences with a definition of the problem and a review of available resources. Some pointers are given in **Table 7**. The separation of enantiomers requires special stationary phases and some separations of isomers use liquid crystal stationary phases that are not common laboratory items. Fast separations require special equipment and truly fast separations are only possible for simple mixtures. The separation of complex mixtures can be speed-optimized but not necessarily performed quickly on the same timescale used for simple mixtures. Preparative separations are usually performed with packed columns unless only a small amount of material is to be isolated. Most analytical separations are performed using WCOT and PLOT columns, except for reasons noted earlier. A general guide to the selection of column types and dimensions is given in **Table 8**. For a sample of unknown composition a generic method for sample evaluation is provided in **Table 9**. These instruction sets will work for most simple mixtures and provide a starting point for difficult samples.

Injection and Detection Considerations

The choice of sample inlet depends on the injection volume, concentration of analytes, thermal stability of the analytes, concentration of involatile matrix components, volatility range of the analytes, relative volatility difference between the analytes and

Table 7 Defining the problem and utilizing available resources to formulate a solution

Problem definition
How many detectable compounds are present in the sample? This is the only way to know that a separation is complete. It indicates the complexity of the problem, if only because, statistically, as the number of components requiring separation increases, so does the difficulty of achieving the separation. Fast separations are not easy for complex mixtures
Are all components equally relevant? The only separation required is that of the compounds of interest from each other and all other compounds in the sample. The latter compounds can be considered as matrix components and need not be individually separated. This reduces the difficulty of providing a separation fit for the defined purpose
Are standards available for the compounds of interest? This enables peaks to be tracked through initial trial separations and links difficult-to-separate compound pairs to their structure so that informed changes to the separation system can be made. It is required for calibration if quantification is needed
What is the concentration range of relevant compounds? Trace components may initially be missed because of inadequate dynamic range if only the major components are considered. Particular injection techniques and selective detectors may be required to detect some compounds at anticipated concentrations
Is identification of unknowns required? If unknown compounds are to be identified, retention information alone will be inadequate in most cases. Coupling to mass or infrared spectroscopic detectors is usually required to achieve the desired level of confidence in the result
Resources
Literature describing similar separations Substantial databases of the chromatographic literature in an electronic searchable form are available. Column manufacturers' catalogues contain information on the separation of common sample types and certified columns may be available for mixtures subject to routine analysis. Official methods controlled by regulatory agencies usually specify appropriate columns for the separation
Past experience with similar samples Life is a learning experience and there is no substitute for a good memory. What worked in a previous case for a similar sample is probably a good starting point for the new sample. Colleagues may have an informed opinion based on a different lifetime experience
Availability of certain columns and equipment Resources are restricted to available columns and equipment and an evaluation of whether they will provide the information required is performed. Additional resources may have to be purchased or informed decisions made of the suitability of substituting available for desired resources
Compound information from handbooks Structures, molecular weight, boiling point (or vapour pressure), solubility in common solvents is useful information that can be found in handbooks for many compounds which are identical or similar to the compounds of interest. This is useful for stationary-phase selection, derivatization strategies and detector selection

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Table 8 Guide to the selection of WCOT columns

Column internal diameter

- Use 0.25 mm i.d. columns for normal split and splitless injection unless sample overloading is a problem
- Use 0.32 mm i.d. columns for splitless and on-column injection, especially when injecting large sample amounts
- Use 0.53 mm i.d. columns as a replacement for packed columns, for the separation of samples containing < 30 components, or samples with components spanning a wide concentration range
- Use 0.18 mm i.d. (or less) columns when the maximum efficiency is required and for high speed separations (modifications to standard instruments may be needed)

Film thickness

- Standard film thicknesses are used for most applications (0.25 μm for 0.25 and 0.32 i.d. columns)
- Use thin film columns (0.1–0.25 μm) for solutes of low volatility (e.g. waxes, triglycerides, steroids, etc.)
- Use thick film columns (1–5 μm) for volatile solutes (e.g. solvents, gas-purgeable compounds)
- Choose columns with a similar phase ratio to obtain similar retention (larger phase ratios reduce retention)

Stationary phase

- If the sample composition is unknown, begin with a nonpolar stationary phase such as a poly(dimethylsiloxane) or poly(dimethyldiphenylsiloxane) with a low mol fraction of diphenylsiloxane groups that separate mainly by differences in volatility
- To improve selectivity, choose a stationary phase whose polarity best matches that of the solutes (similar dipolarity or complementary hydrogen bond interactions). See Figure 2 for the systematic identification of suitable stationary phases
- Consider using a PLOT column for the separation of light hydrocarbons and gases (other applications are indicated in Table 6)

Set-up conditions

Internal diameter (mm)	0.18	0.25	0.32	0.53
Flow rate (mL min^{-1})				
Hydrogen, $u = 40$ cm s^{-1}	0.6	1.4	2.4	5.2
Helium, $u = 20$ cm s^{-1}	0.3	0.7	1.2	2.6
Sample capacity (μg)	< 0.05	0.05–0.1	0.4–0.5	1.2
Separation number	40	30	25	15
Separation efficiency (n m^{-1})	5300	3300	2700	1600

solvent, and the required accuracy and precision. Split injection is commonly used for evaluating separation conditions, even if a different injection technique is used for routine applications. Split injection involves offline vaporization and mixing of the sample vapours with the gas phase, a portion of which is the carrier gas flow for the column and is responsible for transporting a fraction of the sample into the column. Sample bands are narrow, preserving the resolving power of the column. Split injection can handle samples containing involatile matrix components and is the preferred method for injecting gases and volatile samples such as solvents. Accuracy and precision are often poor compared to

Table 9 Generic exploratory conditions for the separation of a sample of unknown composition

Stationary phase	Nonpolar poly(dimethylsiloxane) of poly(dimethyldiphenylsiloxane) wiht 5 mol% diphenylsiloxane groups
Column	Length 10–30 m Internal diameter 0.25 or 0.32 mm Film thickness 0.25 μm (1.0 μm for volatile compounds)
Flow rate	u_{opt}
Temperature	Programme from 50 to 300°C at 20°C min^{-1} (or to temperature limit for phase) Note the elution temperature (T_E) and range of T_E values T_E range < 25°C isothermal analysis T_E range > 25°C programmed analysis
Isothermal	Optimize range of retention factors (k). T_{opt} found from plot of log k versus $1/T$
Programmed	From original programme: **1** Select T initial ($T_E - 20$ for first component) **2** Select T final ($T_E + 20$ for last component) **3** Programme rate selected based on complexity. Simple mixture 10°C min^{-1} or higher and complex mixture 1–2°C min^{-1}
Injector/detector Temperature	Initially high (*c.* 350°C). Reset, based on findings in trial chromatograms *c.* 25°C higher than the final column temperature
Injector	Split with a split ratio 1 : 50 to 1 : 100
Detector	Universal (flame ionization detector)

Table 10 Characteristic properties of common detectors

Detector	*Minimum detectable amount*	*Linear response range*	*Selectivity*
Thermal conductivity	3×10^{-9} g mL^{-1}	10^4	
Flame ionization	10^{-12} g s^{-1}	10^6	
Thermionic ionization	10^{-13} g s^{-1}(N) 10^{-14} g s^{-1}(P)	10^4	4×10^4 gC/gN 7×10^4 gC/gP 0.5 gN/gP
Photoionization	10^{-12} g mL^{-1}	10^7	
Helium ionization	4×10^{-14} g s^{-1}	10^4	
Electron capture	10^{-13} g mL^{-1}	10^4	
Flame photometric	10^{-11} g s^{-1} (S) 10^{-12} g s^{-1} (P)	Nonlinear 10^5	10^3–10^6 gC/gS 5×10^5 gC/gP
Sulfur chemiluminescence	4×10^{-13} g s^{-1} (S)	10^3–10^4	10^6–10^7 gC/gS
Microwave plasma	$1–75 \times 10^{-13}$ g s^{-1}	10^4	Large
Electrolytic conductivity	10^{-12} g s^{-1} (N) 10^{-13} g s^{-1} (Cl) 5×10^{-13} g s^{-1} (S)	10^3–10^5	10^4–10^9 gC/g(N, Cl or S)

other injection techniques and sample information is not preserved for mixtures of a wide volatility range. Splitless injection allows larger sample volumes to be injected for trace analysis but requires an effective refocusing mechanism using cold trapping or solvent effects. Optimization of injection conditions is relatively complicated and time-consuming but accuracy and precision are good for favourable cases. On-column injection is the most accurate and precise injection technique but is limited to small sample volumes and requires relatively clean extracts. Programmed temperature vaporization injection is able to emulate all of the above injection methods as well as allowing large volume injections in the solvent-venting mode. Injection techniques using flash vaporization place the greatest thermal stress on the sample and are unsuitable for labile compounds. All methods can be automated, resulting in improved accuracy and precision compared to manual injection techniques.

Gas chromatography is blessed by a number of reliable and near universal and selective detectors (**Table 10**). Interfacing of gas chromatography to spectroscopic detectors for structural elucidation as well as quantification is straightforward and reduced to routine practice. For general applications the flame ionization detector is difficult to eclipse. It is sensitive, rugged, has a wide linear range, and it has a near universal response to carbon-containing compounds. It has a poor response to the noble gases and certain simple organic compounds containing a single carbon atom bonded to nitrogen, oxygen or sulfur. Thermal conductivity or helium ionization detection can be used for these compounds. A wide range of element-selective detectors and structure-selective detectors has been developed for particular applications demanding matrix discrimination, low sample detectability, or for portable instruments. There are few situations encountered in gas chromatography where the identification of a suitable detector is the limit to progress.

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Further Reading

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